Relationship between chronic inflammation and depression.

Search for biomarkers and possible drug targets

Universitat Autònoma de Barcelona

Germans Trias i Pujol Hospital

Autor: Clara Boter Carbonell

Tutor: Eva M. Martínez Cáceres.
Professor of Immunology at HGTP.

University: Universitat Autònoma de Barcelona
Hospital Germans Trias i Pujol

31/05/2016

Relationship between chronic inflammation and depression.

Search for biomarkers and possible drug targets

0. ABSTRACT

It has been predicted that depression will become the second-leading cause of disability worldwide after human immunodeficiency virus (HIV) in 2030[1]. Numerous recent data suggest an important role of inflammation in patients with depressive disorder. In fact, there has been found an increase of inflammatory markers in some depressed patients,[2,3,4,5] and the administration of pro-inflammatory cytokines or their inducers has been shown to cause symptoms of depression.[2] Besides this, it has been discovered that patients with chronic inflammatory diseases (rheumatoid arthritis, psoriasis, inflammatory bowel disease ...) show a higher prevalence of depression.[6]

Therefore, we hypothesize that manifestations of depression are related to chronic inflammation, at least in some patients with major depressive disorder. Thereby, an anti-inflammatory treatment may improve symptoms of depression. The general objective of this study is to analyse the relationship between inflammation and major depressive disorder (MDD) and search for potential diagnostic biomarkers and therapeutic targets.

INDEX

1. BACKGROUND

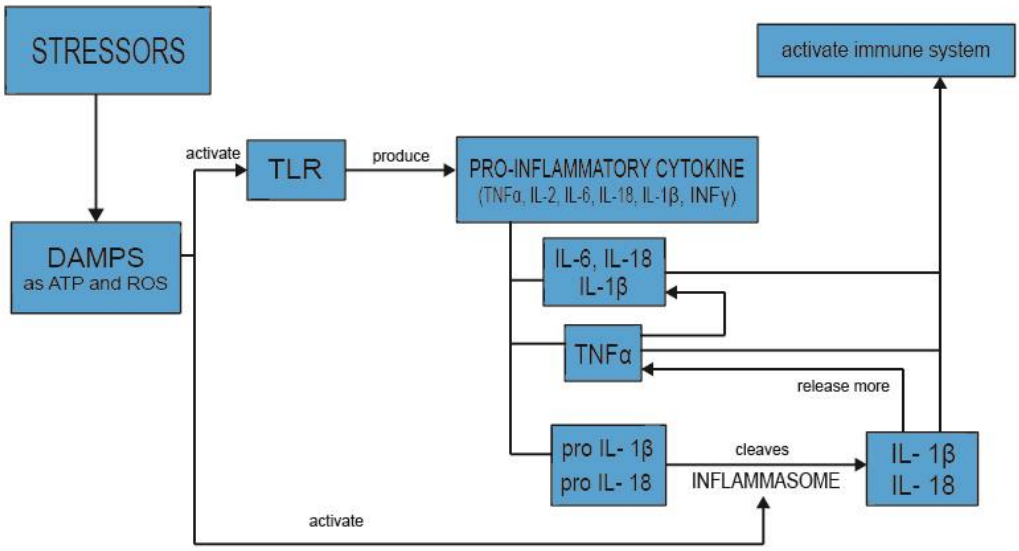
1.1 INTRODUCTION:

Recent studies show that it is possible to understand the development of depression in some patients by dysregulated and prolonged immune activation.[3,4] The behavioural changes may be caused by an inflammatory response that leads to a mirrored immune response within CNS (Neuroinflammation).[7]

Activation of the immune system is the body natural reaction to infection or tissue damage.[4] Actually, depression shares many characteristics with Sickness behaviour*: anhedonia, fatigue, psychomotor slowing, decreased appetite, sleep alterations…[5,8] Indeed, up to 50% of patients with chronic systemic conditions (e.g., cardiovascular disease, autoimmune disease, and cancer) show a clinic depression.[9]

However, since nowadays psychological and physical stressors have been related to depression, recent studies evidence that acute and chronic stress may activate the innate immune system.[10]

1.2 IMMUNE SYSTEM ACTIVATION BY STRESS:



Stressors can activate the immune system by the production of DAMPs* (Damage-associated molecular pattern) as ATP* and Reactive Oxygen and Nitrogen Species (RO&NS). These molecules are able to stimulate the Toll-Like Receptors* (TLR) leading the production of pro-inflammatory cytokines.

The most important pro-inflammatory cytokines, released by the innate immunity, with the ability to alter behaviour are TNF-α, IL-1β, IL-6.[7,11]

Additionally, DAMPs induce the formation of a large multiprotein aggregate containing Nod-Like Receptors (NLR) and mature caspase-1 (Inflammasome*). The most studied Inflammasome is the NLRP3 which cleaves pro-IL-1β and pro-IL18 into mature IL-1β and IL-18, allowing their release from the cell.[2,12,11]

Released IL-1β is thought to induce TNFα release because the increase in IL-1β precedes the increase in TNFα.[12] IL-1β and TNFα induce the inflammatory response and activate the adaptive immune system in the periphery[11]. Furthermore, TNFα induces the release of a variety of pro-inflammatory cytokines such as IL-6, IL-8 and IL-1β by stimulated macrophages.[1,2,7]

Different models for Inflammasome activation by DAMPs have been proposed.[11] One way is dependent on the stimulation of the purinergic type 2X7 receptors (P2X7*) by ATP*.[13,9] This receptors have been found in microglial and blood cells.

The mechanisms by which stress produces ATP is not completely understood. It is thought that stress leads to an increase of glutamate at the hippocampus, which in turn stimulates the release of ATP from astrocytes by a Ca^{2+} -dependent mechanism . Other sources of ATP acting on microglial P2X7R are damaged or dying cells, which also can be induced by stress.[12]

In addition, all DAMPs and PAMPs, including ATP and particulate/crystalline activators, induce the generation of reactive oxygen species (ROS). A ROS-dependent pathways also triggers NLRP3 Inflammasome complex formation.[11]

The Inflammasome is one of the most important possible drug target for depression. Several clinical studies use as objectives the upstream signalling, such as P2X7R antagonist, antioxidants, and Redox cell control by glutathione-increasing therapy. Glutathione* is a crucial component of cellular antioxidant defences and plays an important role in detoxification of ROS and RNS and their toxic metabolites such as lipid peroxides.[14] Other studies try to target the downstream signalling, including caspase-1 inhibitors, anti-IL-1β therapy, TNF-α inhibitors and recombinant IL-1Ra[15]

For instance, it is found in rodents that the release of IL-1β is blocked by pre-treatment with P2X7 antagonist[12] and by The Inflammasome inhibitor VX-765. As a result, decreased levels of interleukin-1β significantly moderated the depressive-like behaviours induced by chronic mild stress in these animals.[13]

1.3 HOW DOES THE IMMUNE SYSTEM REACH THE BRAIN?

As presented before, the behaviour changes may be developed by chronic Neuroinflammation. Even the activation of the immune system starts in the periphery, inflammatory signals may access the brain by humoral, neural and/or cellular pathways.

Humoral pathway**:** Pro-inflammatory cytokines access the brain through structures in which there is no blood-brain-barrier (BBB).[3,5] However, it can also bind to saturable transport molecules on the BBB [2]

Neural pathway: Pro-inflammatory cytokines stimulate primary afferent nerve fibers in the vague nerve which transfer information to brain areas. [3, 5, 16] In a normal situation vague nerve suppresses innate immunity and pro-inflammatory cytokine production. However, the pronounced sympathovagal imbalance counteracts this mechanism, and consequently induces the maintenance of a chronic inflammation.

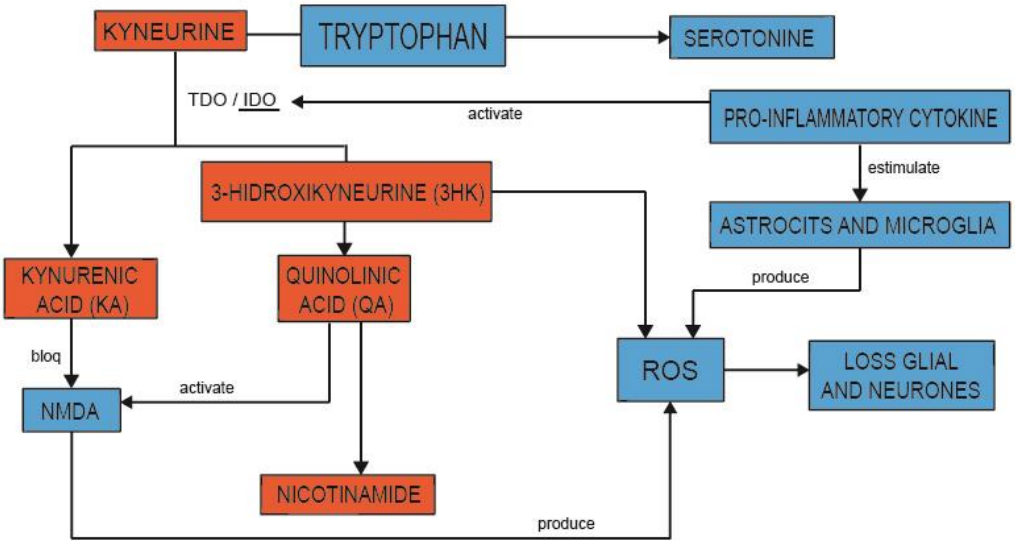
Cellular pathway: Pro-inflammatory cytokines, notably TNF-α, are able to stimulate microglia to produce monocyte chemoattractant protein-1 (MCP-1) and astrocytes in order to produce chemokines such as CCL2 and CXCL1. These induce the recruitment of monocytes into the brain (parenchyma and vasculature).[5]

1.4 POSSIBLE MECHANISMS BY WHICH CYTOKINES AFFECT BEHAVIOUR:

Reduction of synaptic availability for monoamines: IL-1β and TNFα have been shown to increase the expression and function of the reuptake pumps of serotonin.[2] In addition IFN-α decreases the expression of serotonin receptor 1A.[3]

Excitotoxicity: Tryptophan is an essential amino acid used in the brain for the synthesis of serotonin.[3] Usually, it is catabolized by the tryptophan dioxygenase enzyme (TDO) in the liver, but tryptophan oxidation can also occur extrahepatic by the Indoleamine 2,3 dioxygenase enzyme (IDO).[3] IDO-induced degradation normally is negligible but is highly inducible by pro-inflammatory cytokines. [3,5,10,17]

Both enzymes degrade tryptophan along the kynurenine pathway. Kynurenine can be metabolized into two catabolic branches, leading to Quinolinic acid (QA) or Kynurenic acid (KA).[3] QA, is an NMDA receptor agonist, which stimulate glutamate release and block glutamate reuptake by astrocytes.[2] In contrast, KA is an NMDA receptor antagonist, and could be neuroprotective. [3]



In addition, 3-Hydroxykynureine (3-HK) another metabolite in the Kynurenine degradation generates free radical species that cause oxidative stress and lipid peroxidation.[3,10]

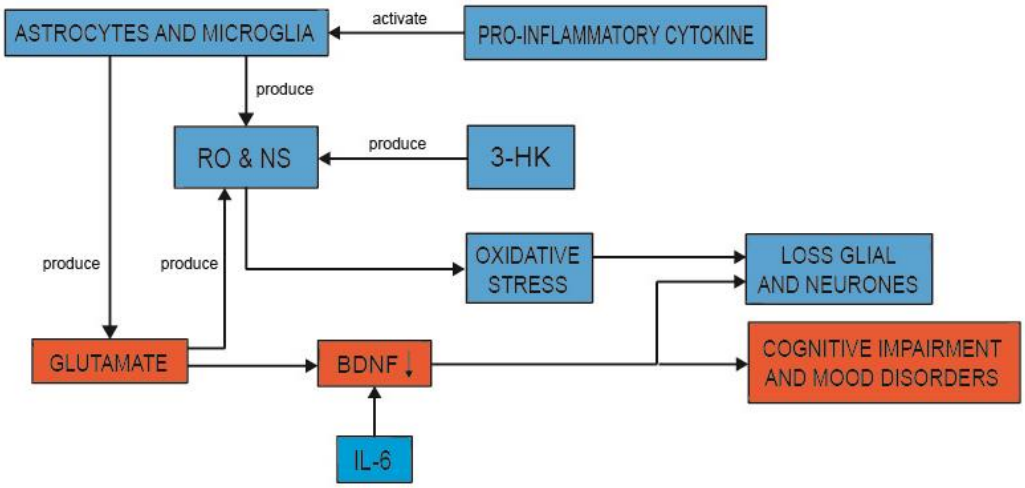
Pro-inflammatory cytokines can also stimulate astrocytes and microglia to release reactive oxygen and nitrogen species that, in combination with 3-HK, can amplify oxidative stress. As a consequence, it can contribute to the loss of glial and neuronal cells in multiple mood-relevant brain regions.[5,10]

Through the generation of reactive oxygen and nitrogen species (RO&NS), inflammatory cytokines have also been found to decrease the availability of tetrahydrobiopterin (BH_4), a key enzyme co-factor in the synthesis of Dopamine[2] which is also required for NO synthesis (a metabolite of RO&NS generation)[5]

Moreover, the excessive release of glutamate by astrocytes can access extra synaptic NMDA receptors, which mediate excitotoxicity and decrease the production of trophic factors including brain-derived neurotrophic factor (BDNF). [5]

The decrease of BDNF induced by glutamate and IL-6, alters the neural connectivity, produces alterations in the emotional responses and leads to impaired neuroplasticity and

decreased neurogenesis (particularly in the hippocampus), which have been associated with the origin of cognitive impairment and mood disorders[10, 18].



Effects on neuroendocrine function: A rich literature has demonstrated that acute administration of pro-inflammatory cytokines and cytokine inducers can activate the hypothalamic-pituitary-adrenal axis (HPA-axis).[3, 5, 13] Chronic cytokine exposure may influence HPA axis through inhibitory effects on the cortisol receptor.[5] Cytokines can also decrease GR alpha (active form of the receptor) and increase GR beta (inert GR isoform) which reduces the sensitivity of GRs to cortisol.[5,10] Given the role of endogenous glucocorticoids in inhibiting inflammatory responses, cytokine-induced glucocorticoid resistance can exacerbate the uncontrolled inflammation.[5]

1.5 ROLE OF PRO-INFLAMMATORY CYTOKINES:

IL-1β exposure was widely reported to cause depressive behaviours and suppression of locomotor activity and social exploration.[13,12] However, in a cumulative meta-analysis, it wasn't neither confirmed nor refuted the association between **IL-1β** and MDD.[19] The lack of association could be due to the low concentrations of IL-1β in the blood, which makes more difficult to determine the amount using conventional immunological assays, [20,19]

The same meta-analysis, confirmed higher levels of **IL-6** and **CRP*** in the blood of patients with major depression compared to non-depressed controls at 5 years follow-up.[19] Nevertheless, no cross-sectional association was found between IL-6 and CRP with depressive symptoms at baseline.[21]

In this meta-analysis, the association between **TNF-α** and MDD was less convincing. Nonetheless, during TNFα therapy depressive symptoms can occur as a secondary effect, which suggests the existence of potential confounders modulating the association between TNFα and MDD.[19] Furthermore, in animal studies, TNFα administration has found to cause depressive-like symptoms.[1]

Anti-TNFα drugs are very effective in the treatment of chronic inflammatory and autoimmune disorders such as Rheumatoid arthritis, inflammatory bowel disease or Psoriasis. Recent clinical trials* have shown that anti-TNFα are effective in decreasing depressive symptoms associated with these disorders. A systematic review has already revealed that anti-TNFα drugs have an antidepressant effect and can improve the antidepressant response of other drugs.[1, 22]

2. HYPOTHESIS:

Manifestations of depression are related to chronic inflammation, at least for some patients with Major Depressive Disorder (MDD). Thereby, an anti-inflammatory treatment may improve symptoms of depression in those patients.

3. MAIN OBJECTIVE

To analyse the relationship between inflammation and Major Depressive Disorder (MDD).

4. SECONDARY OBJECTIVES:

1. To analyse the levels of inflammatory biomarkers in patients with an episode of MDD and to compare them against a healthy control group.
   1.1. To assess if different grades of severity of depressive disorders are associated with different inflammatory profiles.
2. To identify possible pharmacological targets which may help to ameliorate the symptoms of depression.
3. To analyse the effect of anti-TNFα treatment on the severity of the depressive disorders and immunological profile.

5. OBJECTIVE 1: TO COMPARE THE LEVELS OF INFLAMMATORY BIOMARKERS BETWEEN PATIENTS WITH AN EPISODE OF MDD AND HEALTHY CONTROL.

5.1 DESIGN OF THE STUDY:

Observational cross-sectional, case control study.

5.2 METHODS

The study will compare serum cytokine levels between patients with major depressive disorder and healthy individuals. Additionally, possible patterns of cytokine expression and severity of depression will be searched.

5.3 STUDY POPULATION:

Since the variables studied have no history of normality assumptions, based upon previous studies, we will select 50 patients in each group for the study. This is the minimum number necessary to perform a multivariate analysis with 5 independent variables. Moreover, in this way it will be possible to apply the central limit theorem, which states that above 30 sample size is enough for having good reliability in the analysis of the variables in relation to normality.

The depressed group will include up to 50 patients with a new diagnosis of a Major Depressive Disorder episode attending at the Psychiatric Department of Hospital Germans Trias i Pujol.

The diagnosis of depression will be established by using the Structured Clinical Interview for DSM-5 (SCID-5). It is an interview guide for making DSM-5 diagnoses by a clinician or trained mental health professional that is familiar with the DSM-5 classification and diagnostic criteria.

The control group will consist of healthy volunteers without depression (hospital and/or university employees).

Exclusion criteria (for both cohorts):

- Pregnancy.
- Individuals under antidepressant or antipsychotic treatment.
- Individuals with autoimmune or infectious diseases.

- Individuals under immunosuppressive drugs or immune system modifiers (e.g. INFα or INFβ treatment).
- Individuals with chronic systemic diseases.
- Individuals with diabetes, obesity or metabolic syndrome.
- Individuals with Alzheimer disease or with other cognitive problems.
- Individuals with chronic pain or fatigue.
- Individuals younger than 25 years or older than 60 years.

All participants should have signed the informed consent before entering at the study.

5.4 VARIABLES

Possible confounding variables collected in the interview:

- Age
- Gender
- Body Mass Index (BMI)
- Socioeconomic status
- Race

Psychopathological variables:

-Interview SCDI-V (Structures Clinical Interview for DSM-V) applied to the diagnosis of major depressive disorder.

- Montgomery-Asberg Depression Rating Scale (MDRS) to measure depressive clinical severity. [23]

Biochemical variables analysed. Inflammatory biomarkers.

- IL-1β
- TNF-α
- IL-6
- CRP

5.5 DATA COLLECTION:

At baseline, within a period not exceeding one week from the diagnosis of depression, the MDRS to assess the severity of depression will be applied to the selected individuals and a specific interview will be designed to cover all the possible confounding variables.

In addition, as the level of cytokines is highly variable, blood samples will be drawn three times every other day during one week for analysing the inflammatory biomarkers. The average of these results will be used as the statistical value to compensate the variability of cytokine levels.

Briefly, peripheral blood will be collected from patients and healthy volunteers at the same time period (8-10 a.m. in fasting conditions). Peripheral blood will be kept 2 h at 4 ºC and centrifuged 10 min at 1800 rpm to obtain cell-free serum. At least 5 aliquots of serum of each time point will be collected and kept frozen at -80ºC until use.

Serum concentrations of IL-1β, IL-6 and TNF-α will be determined using a human cytokine ELISA test.[24]. The Enzyme Linked Immunosorbent Assay (ELISA) is a powerful method for detecting and quantifying a specific protein in a complex mixture. The method enables analysis of protein samples immobilized in microplate wells using specific antibodies.[25]

CRP will be analysed by turbidimetry, a method which involves measurement of the intensity of light transmitted through a medium.[26] When PCR reacts with an specific antibody, in the presence of polyethylene glycol, precipitating immune complexes are formed which cause turbidity. This is detected by the turbidimetry and is related to the concentration of PCR.

5.6 STATISTICAL ANALYSIS OF THE RESULTS

After obtaining the average levels for each patient, the main values of each group will be compared. If the quantitative variables follow a normal distribution the T-student test will be applied, if not, the parametric test Mann Whitman will be used.

Eventually, a bivariate analysis of data obtained according to the confounding variables will be done.

Additionally, once the biomarkers results will be obtained, a correlational study between severity score on the Montgomery test and the inflammatory biomarkers levels will be sought applying the ANOVA statistical multiple regression model.

5.7 CHRONOLOGY:

Month 1-3: Will serve to ask for authorization of the Ethics Committee of the hospital, to set up the protocols and to prepare the questionnaires.

Month 3-12: Recruitment of the study individuals. Once the subjects have been selected, the blood drawn of the both groups will start in less than one week to avoid leaving patients too long without starting treatment.

Days	Recruitment period (maximum 1 week)	0	1	2	3	4	5	6
Diagnosis of Depression	X							
Check inclusion criteria	X							
Give information to the patient	X							
Deliver informed consent	X							
Signed informed consent		X						
MDRS questionnaire		X						
Blood drawn			X		X		X	

6. OBJECTIVE 2: TO IDENTIFY POSSIBLE PHARMACOLOGICAL TARGETS WHICH MAY HELP TO AMELIORATE THE SYMPTOMS OF DEPRESSION.

6.1 DESIGN OF THE STUDY:

A pilot experimental design with paired samples will be done. In this, the samples from each individual will be analysed in five different conditions.

6.2 METHODS

The 15 depressive patients who have the highest rate of inflammatory markers in the first study will be selected to analyse the effect of different drugs that block Inflammasome on dendritic cells (DC).

Five DC cultures from each patient will be analysed. DC will be generated from peripheral blood monocytes cultered for 5 days in the presence of GMCSF + IL-4 adding LPS stimulus (100 μg/mL) on day 4. On day 5 all will be washed and the indicated stimulus will be added for 24h:

- DC + placebo (PBC)
- DC + ATP.
- DC + ATP + anti P2X7R ATP.
- DC + ATP + anti-pro-IL-1β.
- DC + ATP + caspase-1 inhibitor.

After the incubation period, the effectiveness of the various drugs against Inflammasome will be analysed by comparing the amount of IL-1β secreted in the supernatant.

6.3 STUDY POPULATION:

Although there is not enough information from prior studies to calculate with precision the sample size, from similar studies of the literature it is estimated that a sample size of 15 patients will be enough.

6.4 VARIABLES

The IL-1β will be the only variable analysed.

6.5 DATA COLLECTION:

Levels of IL-1β from culture supernatant of each sample will be evaluated using a human cytokine ELISA test.[24].

6.6 STATISTICAL ANALYSIS

The results will be compared using an analysis of variance for repeated measures.

6.7 CHRONOLOGY

For the study three months for the preparation of the protocols and twelve months for the analysis of the cultures will be needed.

7. OBJECTIVE 3: TO ANALYSE THE EFFECT OF ANTI-TNF TREATMENT ON THE SEVERITY OF THE DEPRESSIVE DISORDERS AND IMMUNE PROFILE.

7.1 DESIGN OF THE STUDY:

A descriptive, prospective and longitudinal pilot study will be executed.

7.2 METHODS

The relationship between markers of inflammation and severity of depression will be analysed in patients with co-diagnosis of rheumatoid arthritis or psoriasis and resilient depression before starting the anti-TNF-α treatment and at 4, 12 and 24 weeks.

7.3 STUDY POPULATION:

There is not enough information from prior studies to calculate with precision the sample size. Nevertheless, it is estimated that follow up of 15-20 patients is sufficient for this exploratory study.

Inclusion criteria:

Diagnosis of Rheumatoid arthritis or Psoriasis (10 individuals of each one) attending at the Dermatological Department of Hospital Germans Trias i Pujol who have to start taking anti-TNF therapy[27] and who also have diagnosis of depression with resistance to antidepressant drugs.

All participants should have signed the informed consent before they entered the study.

Exclusion criteria:

- Pregnancy.
- Individuals under antidepressants or antipsychotic treatment.
- Individuals with another autoimmune, infectious diseases or systemic disease.
- Individuals under immunosuppressive drugs or immune system modifiers (e.g. INFα).
- Individuals with diabetes, obesity or metabolic syndrome.
- Individuals with Alzheimer disease or with other cognitive problems.
- Individuals with chronic pain or fatigue.
- Individuals younger than 25 years or older than 60 years.

7.4 VARIABLES

Psychopathological variables:

-Interview SCDI-V (Structures Clinical Interview for DSM-V) applied to the diagnosis of major depressive disorder.

- Montgomery-Asberg Depression Rating Scale (MDRS) to measure depressive clinical severity. [23]

Biochemical variables analysed. Inflammatory biomarkers.

- IL-1β
- IL-6
- TNF-α
- CRP

Clinical parameters for patients suffering from Rheumatoid Arthritis

- Rheumatoid Arthritis Quality of Life (RAQoL)[28] to assess the quality of life of patients.
- Disease Activity Score 28 (DAS28) to measure rheumatoid arthritis (RA) disease activity, to determine whether the signs and symptoms have reduced or stopped, and if treatment needs to be adjusted.[29]

Clinical parameters for patients suffering from Psoriasis:

- The Psoriasis Disability Index (PDI) as a tool for assessment of quality of life in psoriasis.[30]

7.5 DATA COLLECTION:

The patients will be assessed for psychosocial, immunological biomarkers and clinical parameters at baseline and 4, 12, 24 weeks after starting anti-TNF therapy. For this purpose, the MDRS, RAQoL and DAS28 or PDI questionnaires will be performed. The analyse of cytokines and the used methods will be the same like in the previous study.

7.6 STATISTICAL ANALYSIS OF THE RESULTS

At the end of the study the results of the severity score, clinical parameters and the level of cytokines of each individual will be compared by an analysis of the variability in repeated measures.

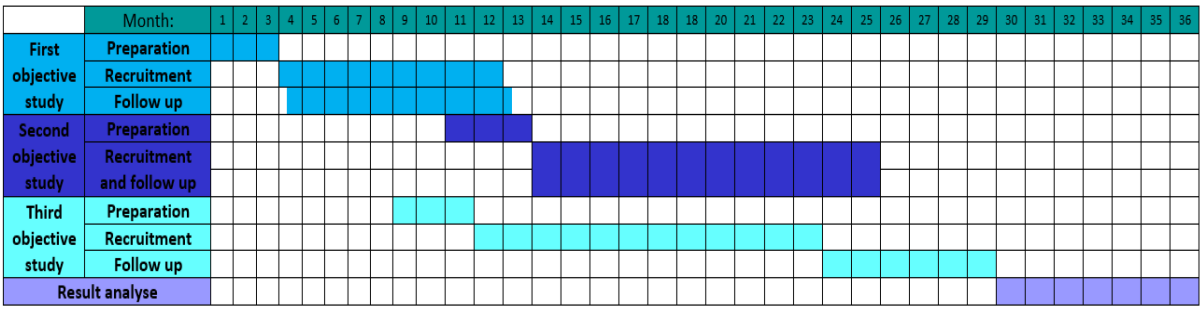
7.7 CHRONOLOGY OF THE STUDY

The study inclusion period will last 12 months. Once subjects will be selected the interview and blood test will be done before starting the therapy, at week 4, 12 and 24. Therefore, the follow-up period of all patients, including the last included, will be until 15th month.

WEEKS	Recruitment period (maximum 1 week)	0	4	12	24
Check inclusion criteria	X				
Give information to the patient	X				
Deliver informed consent	X				
Signed informed consent		X			
Clinical Test		X	X	X	X
MDRS questionnaire		X	X	X	X
Blood drawn		X	X	X	X

8. STUDY DURATION

The complete study will last three years (36 months). The chronology of the study is as follows:



9. DISSEMINATION OF RESULTS.

The results of this study will be presented in national and international congresses of Immunology and Psychiatry and a review paper and at least one original peer-reviewed will be done.

Furthermore, presentations to patient associations and seminars at IGTP will be organized.

10. STUDY LIMITATIONS

Objective 1:

It is possible to find a self-selection bias because the characteristics of the group of patients may not correlate with the control group especially in the socioeconomic status. For this reason, a multivariate data analysis with possible confounders will be done.

Another important limitation of this study is the variability of cytokines in blood and the variety of factors that can influence it. For this reason, in the study we will use the average of several samples taken at the same time of the days, in order to reduce the variability. Given that patients cannot stay long untreated for ethical reasons, all samples should be taken the first week after recruitment. In addition, the blood level of various cytokines might be very low, which would make more difficult find significant variation in the sample.

Another measure to prevent the variation of cytokines by other factors, is the exclusion of the study people with mental illness or other systemic diseases. However, this would

make more difficult the recruitment because depression is highly associated with other diseases.

Another problem is to assess the severity of depressive symptoms because it is a subjective parameter. For this reason, we will use the MDRA questionnaire, which according to various systematics reviews, is the most reliable.

Objective 3

Recruitment patients that are going to start anti-TNFα with co-diagnosis of depression should be enough based on the current amount of patients attended at the HGTP. However, it can be difficult to reach 20 subjects for the study. In case, it will not be enough the recruitment period will be extended for six months more.

11. EXPECTED RESULTS

It is expected that patients with depression have elevated inflammatory markers compared to non-depressed subjects. As a result, an improvement in the severity of depression in subjects taking TNF-α is expected.

Referred to in vitro analysis of drugs, it is expected to find a reduction in the production of IL-1β in all the stimulated cultures in which the drug has been added compared to no drug stimulated culture.

12. IMPACT OF THE STUDY FOR THE NATIONAL HEALTH SYSTEM

Depression is one of the most prevalent diseases worldwide. Currently there are only symptomatic treatments with high resistance rate. With the discovery of new drug targets, it is possible to find a better treatment allowing a better management of the disease. This could improve the quality of life for patients, their families and society as a whole; while costs would be reduced as it will reduce recovery time, medical visits and absenteeism.

Furthermore, as stated in several studies, chronic inflammation is the cause of multiple pathologies. Therefore, an effective treatment could help patients affected by other diseases to improve their quality of life.

13. ETHICAL QUESTIONS

First of starting the study, it will be evaluated by the local ethic committees and will be carried out in accordance with the declaration of Helsinki.

14. ACKNOWLEDGMENTS

To carry out this work I want to thank the people who helped me in the elaboration of it:

- Eva M. Martinez Caceres, professor of Immunology at Hospital Germans Trias I Pujol and tutor of the labour.
- Lluís Jover Armengol, professor of biostatistics at the UB.
- Crisanto Díez Quevedo, Doctor in the psychiatric department of Hospital Germans Trias i Pujol.
- Mª José Mansilla, Doctor in Biotechnology.

15. BIBLIOGRAPHY

1. Ma K, Zhang H, Baloch Z. Pathogenetic and Therapeutic Applications of Tumor Necrosis Factor-α (TNF-α) in Major Depressive Disorder: A Systematic Review. *Int J Mol Sci*. 2016;17(5). doi:10.3390/ijms17050733.

2. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol*. 2016;16(1):22-34. doi:10.1038/nri.2015.5.

3. Dantzer R, Connor JCO, Freund GG, Johnson RW, Kelley KW. NIH Public Access. 2010;9(1):46-56. doi:10.1038/nrn2297.From.

4. Friedrich MJ. Research on Psychiatric Disorders Targets Inflammation. *JAMA*. 2014;312(5):474. doi:10.1001/jama.2014.8276.

5. Lucile Capuron (Laboratory of Nut. NIH Public Access. *Pharmacol Ther*. 2012;130(2):226-238. doi:10.1016/j.pharmthera.2011.01.014.Immune.

6. Celińska-Löwenhoff M, Musiał J. [Psychiatric manifestations of autoimmune diseases--diagnostic and therapeutic problems]. *Psychiatr Pol*. 46(6):1029-1042. http://www.ncbi.nlm.nih.gov/pubmed/23479944. Accessed May 20, 2016.

7. McCusker RH, Kelley KW. Immune-neural connections: how the immune system's response to infectious agents influences behavior. *J Exp Biol*. 2013;216(Pt 1):84-98. doi:10.1242/jeb.073411.

8. Shattuck EC, Muehlenbein MP. Human sickness behavior: Ultimate and proximate explanations. *Am J Phys Anthropol*. 2015;157(1):1-18. doi:10.1002/ajpa.22698.

9. Duric V, Clayton S, Leong ML, Yuan L-L. Comorbidity Factors and Brain Mechanisms Linking Chronic Stress and Systemic Illness. *Neural Plast*. 2016;2016:5460732. doi:10.1155/2016/5460732.

10. Tafet GE, Nemeroff CB. The Links Between Stress and Depression: Psychoneuroendocrinological, Genetic, and Environmental Interactions. *J Neuropsychiatry Clin Neurosci*. November 2015:appi.neuropsych. doi:10.1176/appi.neuropsych.15030053.

11. Kuby. *Immunology*. Vol XXXIII.; 2012. doi:10.1007/s13398-014-0173-7.2.

12. Iwata M, Ota KT, Li X-Y, et al. Psychological Stress Activates the Inflammasome via Release of Adenosine Triphosphate and Stimulation of the Purinergic Type 2X7 Receptor. *Biol Psychiatry*. 2015;(13):1-11. doi:10.1016/j.biopsych.2015.11.026.

13. Zhang Y, Liu L, Liu YZ, et al. NLRP3 inflammasome mediates chronic mild stress-induced depression in mice via neuroinflammation. *Int J Neuropsychopharmacol*. 2015. doi:10.1093/ijnp/pyv006.

14. Morris G, Anderson G, Dean O, et al. The Glutathione System: A New Drug Target in Neuroimmune Disorders. *Mol Neurobiol*. 2014:1059-1084. doi:10.1007/s12035-014-8705-x.

15. Yao W, Zhang J-C, Dong C, et al. Effects of amycenone on serum levels of tumor necrosis factor-α, interleukin-10, and depression-like behavior in mice after lipopolysaccharide administration. 2015. doi:10.1016/j.pbb.2015.06.012.

16. Abboud FM, Abboud F, Abboud F, et al. The Walter B. Cannon Memorial Award Lecture, 2009. Physiology in perspective: The wisdom of the body. In search of autonomic balance: the good, the bad, and the ugly. *Am J Physiol Regul Integr Comp Physiol*. 2010;298(6):R1449-R1467. doi:10.1152/ajpregu.00130.2010.

17. Martin-Subero M, Anderson G, Kanchanatawan B, Berk M, Maes M. Comorbidity between depression and inflammatory bowel disease explained by immune-inflammatory, oxidative, and nitrosative stress; tryptophan catabolite; and gut-brain pathways. *CNS Spectr*. 2016;21(2):184-198. doi:10.1017/S1092852915000449.

18. Furtado M, Katzman M a. Examining the role of neuroinflammation in major depression. *Psychiatry Res*. 2015;229:27-36. doi:10.1016/j.psychres.2015.06.009.

19. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimäki M. Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun*. 2015;49:206-215. doi:10.1016/j.bbi.2015.06.001.

20. Chapman P, Reyes C, Gupta V. Normal Physiological Levels of Human Cytokines Using Bio-Plex Pro™ Cytokine Assays. *Bull Bio-Rad Lab*. 2010. http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6029.pdf. Accessed May 13, 2016.

21. Zalli A, Jovanova O, Hoogendijk WJG, Tiemeier & H, Carvalho LA. Low-grade inflammation predicts persistence of depressive symptoms. doi:10.1007/s00213-015-3919-9.

22. Abbott R, Whear R, Nikolaou V, et al. Tumour necrosis factor-α inhibitor therapy in chronic physical illness: A systematic review and meta-analysis of the effect on depression and anxiety. *J Psychosom Res*. 2015;79(3):175-184. doi:10.1016/j.jpsychores.2015.04.008.

23. Description of the Hamilton Depression Rating Scale (HAMD) and the Montgomery-Asberg Depression Rating Scale (MADRS).

24. Luchting B, Heyn J, Woehrle T, et al. Differential expression of P2X7 receptor and IL-1β in nociceptive and neuropathic pain. *J Neuroinflammation*. 2016;13(1):100. doi:10.1186/s12974-016-0565-z.

25. Thermo Scientific. *ELISA Technical Guide and Protocols*. Vol 65.; 2010.

26. A.K.Srivastava. Instrumental Approach to Chemical Analysis. *S Chand Publ*. 2009.

27. Técnica F, Las ORDE, Del C. Ficha técnica REMICADE. *Aemps*. 2014:1-41. doi:10.1158/0008-5472.SABCS12-S5-3.

28. Tijhuis GJ, Jong Z de, Zwinderman AH, et al. The validity of the Rheumatoid Arthritis Quality of Life (RAQoL) questionnaire. *Rheumatology*. 2001;40(10):1112-1119. doi:10.1093/rheumatology/40.10.1112.

29. Fransen J, van Riel PLCM. The Disease Activity Score and the EULAR response criteria. *Clin Exp Rheumatol*. 23(5 Suppl 39):S93-S99. http://www.ncbi.nlm.nih.gov/pubmed/16273792. Accessed May 25, 2016.

30. Lewis VJ, Finlay AY. Two decades experience of the Psoriasis Disability Index. *Dermatology*. 2005;210(4):261-268. doi:10.1159/000084748.

31. Tyring S, Gottlieb A, Papp K, et al. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet*. 2006;367(9504):29-35. doi:10.1016/S0140-6736(05)67763-X.

32. Bassukas ID, Hyphantis T, Gamvroulia C, Gaitanis G, Mavreas V. Infliximab for patients with plaque psoriasis and severe psychiatric comorbidity. *J Eur Acad Dermatol Venereol*. 2008;22(2):257-258. doi:10.1111/j.1468-3083.2007.02310.x.

33. Menter A, Augustin M, Signorovitch J, et al. The effect of adalimumab on reducing depression symptoms in patients with moderate to severe psoriasis: a randomized clinical trial. *J Am Acad Dermatol*. 2010;62(5):812-818. doi:10.1016/j.jaad.2009.07.022.

34. Lichtenstein GR, Bala M, Han C, DeWoody K, Schaible T. Infliximab improves quality of life in patients with Crohn's disease. *Inflamm Bowel Dis*. 2002;8(4):237-243. http://www.ncbi.nlm.nih.gov/pubmed/12131606. Accessed May 20, 2016.

35. Persoons P, Vermeire S, Demyttenaere K, et al. The impact of major depressive disorder on the short- and long-term outcome of Crohn's disease treatment with infliximab. *Aliment Pharmacol Ther*. 2005;22(2):101-110. doi:10.1111/j.1365-2036.2005.02535.x.

36. Minderhoud IM, Samsom M, Oldenburg B. Crohn's disease, fatigue, and infliximab: is there a role for cytokines in the pathogenesis of fatigue? *World J Gastroenterol*. 2007;13(14):2089-2093. http://www.ncbi.nlm.nih.gov/pubmed/17465453. Accessed May 20, 2016.

37. Kekow J, Moots RJ, Emery P, et al. Patient-reported outcomes improve with etanercept plus methotrexate in active early rheumatoid arthritis and the improvement is strongly associated with remission: the COMET trial. *Ann Rheum Dis*. 2010;69(01):222-225. doi:10.1136/ard.2008.102509.

38. Packham JC, Jordan KP, Haywood KL, Garratt AM, Healey EL. Evaluation of Ankylosing Spondylitis Quality of Life questionnaire: responsiveness of a new patient-reported outcome measure. *Rheumatology (Oxford)*. 2012;51(4):707-714. doi:10.1093/rheumatology/ker377.

16. GLOSSARY:

1. **Sickness behaviour**

Sickness behaviour is a behavioural change that could have adaptive benefits[8] for the sick animals/humans in terms of recovery and survivability because it conserves energy for fighting infection and healing wounds, while maintaining vigilance against attack (anxiety).[2]

2. **DAMPs**

DAMPs are components of dead/dying cells and damaged tissues able to trigger the Pattern Recognition Receptors. DAMP include: ATP, heat shock (HSP) and chromatin proteins, uric acid, high mobility group box 1 (HMGB1), amyloid, fragments of extracellular matrix components and molecules linked with oxidative stress (oxidized low density)[11]

3. **TLR:**

TLRs are the most widely studied PRRs are the TLR. These are membrane proteins that recognize and binds to proteins, lipids and nucleic acid molecules on infectious pathogens. The subsequent signalling, activates the innate immune system.

4. **Inflammasome:**

An Inflammasome is a large protein complex of certain Nod Like Receptors (NLR) with other proteins, including key proteases, that activates caspase-1 to generate IL-1 and IL-18. Three NLRs (NLRP1, NLRP3 and NLRC4) have been shown to form Inflammasomes. The best characterized Inflammasome is the NLR3, which is expressed in monocytes, macrophages, neutrophils, dendritic cells and some lymphocytes and epithelial cells but also inside the brain in microglia and invasive macrophages. [11]

5. **P2X7R:**

P2X7R is an ionotropic receptor located predominantly on microglia and macrophages which activates Inflammasome. This receptor is triggered in response to cellular danger signals, such as ATP. [12]

6. **Glutathione:**

Glutathione has important functions in the brain as an antioxidant, and it also acts as a neuromodulator of the glutamate ionotropic receptors, interacts with NMDA receptors, and protects against glutamate excitotoxicity. Glutathione depletion enhances the transcription and damaging effects of these cytokines and predisposes to a pro-inflammatory environment compromising synaptic plasticity, memory, and learning. Lowered activity of the glutathione system is observed in neuro-immune disorders, including depression, ME/CFS, and Parkinson's disease.[14]

7. ATP

ATP is a "warning molecule" for microglial activation and an endogenous agonist of P2X7R receptor of the Inflammasome. Moreover, extracellular ATP is used as a gliotransmitter for astrocytes, but it is also thought to be a neurotransmitter.

8. CRP

CRP (C-reactive protein) is an opsonin which recognizes phosphorylcoline and carbohydrates on bacteria, fungi and parasites, and is then bound by Fc receptors (FCRs) for IgG found on most phagocytes. That protein is released by the liver when it is stimulated by inflammatory cytokines. This has been utilized as a biomarker for detecting inflammation, in which levels exceeding 10 mg/L are considered to predict inflammatory states.[19]

9. Pro-inflammatory cytokines:

Cytokine	Sources	Target and effects
IL-1β	– Monocytes – Macrophages – Dendritic cells – NK cells – Non-immune cells (fibroblast, adipocytes, astrocytes and some smooth muscle cells)	– Induction of local inflammation – Systemic effects (fever, acute phase response…) – Stimulation of neutrophil production
TNF-α	– Monocytes – Macrophages – T cells – NK cells – Neutrophils – Fibroblast	– Regulates growth and differentiation of a wide variety of cell types. Cytotoxic – Promotes angiogenesis, bone resorption and thrombotic processes – Supresses lypogenic metabolism – Induction of acute phase proteins (liver)
IL-6	– Macrophages – Endothelial cells – Some T cells and B cells	– Regulates B and T cell function – Induction of acute phase proteins (liver) – Effects on haematopoiesis – Influence adaptive immune system

17. APPENDIX

17.1 RECENT CLINICAL TRIALS

Recent clinical trials have shown that TNFα blockers are effective in decreasing depressive symptoms associated with inflammatory disorders.

Workers	Patients	Treatment	Results
Tyring et al.[31] (2006)	Psoriasis patients	Etanercept	Compared to placebo administration, Etanercept treatment led to an improvement of at least 50% in depression rating scales at week 12.
Bassukas et al.[32] (2008)	Psoriasis patients	Infliximab	Stabilization or improvement of the manifestations of psychiatric morbidity
Menter et al[33] (2010)	Psoriasis patients	Adalimumab	Adalimumab treatment reduced symptoms of depression and improved health-related quality of life (GOL) in addition to improving psoriasis
Lichtenstein et al. [34] (2002)	Crohn disease patients	Infliximab	Significantly improved GOL in patients with active CD, decreasing fatigue, depression and anger
Persoons et al[35] (2005)	Crohn disease patients	Infliximab	Reported the beneficial effects of infliximab on depression and psychological well-being in active CD
Minderhoud et al[36] (2007)	Crohn disease patients	Infliximab	Significantly reduced depression scores and improved the GOL as well as fatigue in a four-week follow-up study
Kekow. COMET study[37] (2010)	Rheumatoid arthritis patients	Etanercept	Early treatment with ETN+MTX leads to significantly greater improvements in multiple dimensions of patient-reported outcomes (PROs) than MTX alone.
Packham et al. ASCEND study[38] (2012)	Ankylosing spondilitis	Etanercept	Improvement during routine clinical practice or following treatment with ETN. Evaluation of Ankylosing Spondylitis Quality of Life (EASi-QoL) domains have superior or comparable responsiveness than comparable measures.

17.2 INFORMED CONSENT

HOJA DE INFORMACIÓN AL PACIENTE/DONANTE

Estudio para conocer la diferencia entre el nivel de marcadores inflamatorios entre controles sanos y pacientes con Episodio Depresivo Mayor. Incluye la determinación de biomarcadores inflamatorios y el cultivo de células de los pacientes en sangre para analizar la eficacia de nuevos posibles fármacos contra la depresión.

Estimado paciente/control,

El objetivo de este documento es resumirle de forma clara y concisa el propósito del estudio en el que le proponemos participar. Este documento puede contener palabras que no comprenda. Si es así, pídale al médico responsable del estudio o al personal que forma parte del mismo que le explique lo que no entienda. Cuando haya comprendido toda la información, y si desea participar en el estudio, se le pedirá que firme un consentimiento informado.

El estudio que estamos realizando tiene como objetivo analizar la inflamación como una de las posibles causas de la depresión y estudiar posibles terapias para el tratamiento de la Depressión. Para ello necesitamos muestras de donantes sanos y pacientes con Depresion que nos permitan poder poner a punto este proyecto.

Retirada del estudio

Su participación en este estudio es totalmente voluntaria. Podrá retirar su consentimiento y abandonar el estudio en cualquier momento, sin tener que dar explicaciones a su médico y sin que por ello se comprometa su tratamiento y atención médica futura.

Su médico también podrá retirarle del estudio en cualquier momento si considera que es lo mejor para usted, o no cumple con los criterios necesarios para participar. Podría ser retirado del estudio si se considera que su participación puede serle perjudicial, si precisa algún tipo de tratamiento no permitido en el estudio, si no sigue las instrucciones del estudio, si es mujer y se queda embarazada, o si se cancela el estudio.

Posibles riesgos e inconvenientes

Los efectos adversos que puede presentar serán los mismos que pudiera con la extracción sanguínea y que no difiere del de cualquier extracción sanguínea de la práctica clínica habitual.

Confidencialidad

El acceso a sus datos clínico-asistenciales y biológicos se realizará guardando la más estricta confidencialidad de forma que no se viole la intimidad personal. Sus datos serán tratados de forma que la información que se obtenga no puede identificarle o asociarse a su persona. De esta forma usted no podrá ser identificado durante el análisis y la presentación de los resultados en publicaciones relacionadas con el estudio. Se le garantiza el estricto cumplimiento de la Ley de protección de datos personales (en España, la Ley 15/1999 De Diciembre de Protección de Datos Personales).

Si usted acepta participar en el estudio, autoriza que además del médico y su equipo, su historia médica pueda ser revisada por el personal autorizado por el Promotor del estudio y por las Autoridades Sanitarias Reguladores.

Compensación

No está previsto el pago de los desplazamientos, ni pago por participar en el estudio.

Resultados

Una vez que el estudio haya concluido y se disponga de los resultados, su médico podrá informarle al respecto si lo desea. Si los resultados del estudio se publican y está interesado en conocerlos, se le proporcionará una copia de la publicación o se le facilitará el acceso a los resultados.

Agradecimiento

Le agradecemos el tiempo dedicado a leer este documento que debe guardar. Por favor, tómese el tiempo necesario antes de decidir si desea participar. Si decide tomar parte en el estudio, deberá firmar dos ejemplares de consentimiento informado y quedarse uno. El otro se quedará archivado en su historia clínica.

Si en cualquier momento durante el estudio le surgiera alguna duda al respecto, o bien caso de alguna urgencia, no dude en ponerse en contacto con:

HOJA DE CONSENTIMIENTO INFORMADO

Estudio para conocer la diferencia entre el nivel de marcadores inflamatorios entre controles sanos y pacientes con Episodio Depresivo Mayor. Incluye la determinación de biomarcadores inflamatorios y cultivo de células de la sangre de los pacientes para analizar la eficacia de nuevos posibles fármacos contra la depresión.

He comprendido la información que se me ha proporcionado y he resuelto dudas que tenía al respecto con el profesional sanitario responsable.

Autorizo al equipo médico que me trata a que me extraiga muestras de sangre y las envíe al Servicio de Inmunología del hospital Germans Trias i Pujol, para su procesamiento, almacenamiento y análisis. También doy mi consentimiento para la realización de los cuestionarios propios del estudio.

Respecto a la utilización tanto de la información clínica de mi historial médico como del material biológico sobrante una vez finalizado el estudio:

Autorizo a que el material biológico sobrante (tratado de forma anónima), y los datos clínicos asociados se utilicen para investigación

SÍ □ NO □

Deseo que se me comunique información importante derivada de la investigación

SI □ NO □

Autorizo a ser contactado en el caso de necesitar más información o muestras biológicas

SI □ NO□

Nombre y apellidos del paciente o representante legal Firma	Nombre y apellidos de la persona que obtiene el consentimiento Firma